THE ROYAL SOCIETY

Received 7 August 2002
Accepted 3 September 2002
Published online 28 November 2002

Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs?

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The activation of an immune response is beneficial for organisms but may also have costs that affect fitness. Documented immune costs include those associated with acquisition of special nutrients, as well as immunopathology or autoimmunity. Here, we test whether an experimental induction of the immune system with a non-pathological stimulant can elevate energy turnover in passerine birds. We injected phytohaemagglutinin (PHA), a commonly used mitogen that activates the cell-mediated immune response, into the wing web of house sparrows, *Passer domesticus*. We then examined energetic costs resulting from this immune activity and related those costs to other physiological activities. We found that PHA injection significantly elevated resting metabolic rate (RMR) of challenged sparrows relative to saline controls. We calculated the total cost of this immune activity to be *ca*. 4.20 kJ per day (29% RMR), which is equivalent to the cost of production of half of an egg (8.23 kJ egg^{-1}) in this species. We suggest that immune activity in wild passerines increases energy expenditure, which in turn may influence important life-history characteristics such as clutch size, timing of breeding or the scheduling of moult.

Keywords: immunity; resting metabolic rate; phytohemagglutinin; house sparrow; energy expenditure

1. INTRODUCTION

Organisms should possess an immune system that immediately recognizes and destroys intruding pathogens and parasites, yet too strong an immune system might generate excessive costs. An immediate, strong response to every non-self particle may be costly in terms of autoimmunity (Råberg *et al.* 1998) or because nutrient and energy resources necessary for immune activity may have to be drawn away from important organismal functions, such as growth, tissue repair or reproduction (Sheldon & Verhulst 1996). Indirect costs of immune activity have been demonstrated by analysing the effects of induced immune activity on fitness parameters (sexually selected ornament elaboration (Zuk & Johnsen 2000); adult survival (Moret & Schmid-Hempel 2000); nestling provisioning rates (Ilmonen *et al.* 2000; Råberg *et al.* 2000); incubation effort (Cichon 2000); and juvenile growth rates (Klasing *et al.* 1987; Fair *et al.* 1999)) or, conversely, by examining the effects of changes in fitness components on immune responses themselves (parental effort (Saino *et al.* 1997; Nordling *et al.* 1998; Deerenberg *et al.* 1997; Moreno *et al.* 1999); reproductive state (Bentley *et al.* 1998); or sexual characters (Saino & Moller 1997)). In almost all cases, immune activity was shown to be costly (reviewed in Sheldon & Verhulst 1996; Klasing & Leshchinsky 1999; Lochmiller & Deerenberg 2000; Norris & Evans 2000).

Existing evidence for the direct mechanisms, particularly energetic costs, underlying these indirect costs of immune activity in non-human vertebrates is currently equivocal. Demas *et al.* (1997) found that the antibody response of laboratory mice (*Mus musculus*) to keyhole limpet haemocyanin (KLH) challenge elevated resting metabolic rate (RMR) by 27%. Similarly, Ots *et al.* (2001) found a 9% increase in basal metabolic rate (BMR) after sheep red blood cell (SRBC) challenge of wild great tits (*Parus major*) in the field. However, Svensson *et al.* (1998) found that antibody response to killed diptheria–tetanus (DPT) virus did not significantly elevate BMR in blue tits (*Parus caeruleus*) kept in aviaries.

Here, we tried to assess the energetic costs of the cell-mediated component of the immune system. We compared RMR of inactive, post-absorptive house sparrows (*Passer domesticus*) challenged with phytohaemagglutinin (PHA) to the RMR of saline-injected birds. We used PHA, a plant lectin that induces T-cell mitogenesis, to induce a delayed-type hypersensitivity response involving local infiltration of tissue by basophils and macrophages (Goto *et al.* 1978). By using this mitogen instead of an experimental infection with a parasite, we could assess the energetic costs associated with an immune response alone, irrespective of the costs that would be imparted by parasite proliferation and subsequent tissue damage and repair (Smits *et al.* 1999). RMR (similar to BMR) represents the 'minimum cost of life', or the metabolic rate of an organism during its inactive phase while thermoneutral and post-absorptive (Aschoff & Pohl 1970; Ricklefs *et al.* 1996). Any elevations in RMR can be attributed to an induced physiological condition, and previous studies have used this technique to assess the costs of many physiological activities including moult, organ growth and egg production (King 1973; Lindstrom *et al.* 1993; Klaasen 1995; Svensson *et al.* 1998).

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We expected that if cell-mediated immune activity was energetically costly, metabolic rate of PHA-challenged individuals (RMR plus immune activity) would be significantly higher than RMR of saline-injected individuals (RMR only). We then used data from other published studies to compare PHA-induced energetic costs to the energetic costs of a variety of other physiological, fitness-related activities in the house sparrow and other passerines. We hypothesize (i) that the activation and use of the cell-mediated part of the immune system is energetically expensive for house sparrows, and (ii) that cell-mediated immune activity is as costly as other physiological activities that impact fitness, such as moult and reproduction (including the generation and maintenance of reproductive organs and production of eggs).

2. MATERIAL AND METHODS

(a) *Animal capture and care*

We captured adult male house sparrows ($n = 14$) with either mist nets or potter traps from agricultural areas in and around Champaign, IL (40° N) during January and February 2000. Before and during the experiments, birds were housed indoors in separate cages under controlled temperature (25 °C), light (12 L : 12 D) and humidity (30% relative humidity) until experiments were performed. For the duration of captivity, all individuals were provided with food (no-waste seed mix) and water *ad libitum*. All birds were maintained in captivity for similar durations before experimentation (around two weeks).

(b) *Immune challenge*

In earlier experiments, we found that previous experience of experimentation affected the metabolic rates of birds; RMR of sparrows on the first night of measurement was higher than during subsequent nights (Martin *et al.* 2000). Thus, to eliminate these effects, we acclimatized birds to the respirometry procedures for two nights before immune challenges were made. Then, at around noon of the third day of the experiment, we injected 100 µl of a 1 mg ml^{-1} solution of crystalline phytohaemagglutinin (PHA-P) purified by affinity chromatography (Sigma 9017) in saline solution (Sigma P3813) into the left wing web of seven birds. In the control trial, we used identical protocols, except that 100 µl of saline solution (from the same stock used as the vehicle for PHA in the treatment group) was injected into the left wing web of seven different sparrows.

To assess the immune response to PHA (or saline), we measured the thickness of the injected wing web to the nearest micrometer using a Starrett gauge (no. 10) following the single wing-web measurement technique, as it reduces handling time and the potential effects of consequent stress on the birds' metabolism (Smits *et al.* 1999). We measured wing-web swelling six times during the experiment: immediately before injection and every evening before respirometry measurements. All measurements were made three times per bird, and because repeatability within individuals was high ($r = 0.97$), we used mean values in all statistical tests (Smits *et al.* 1999).

(c) *Respirometry*

We determined RMR by measuring O_2 consumption and CO_2 production of birds before and after injections in an open flow, push-through respirometry system (Withers 1977). In our system, external air was pumped into a climate-controlled chamber, where it was then channelled through two tubes: one leading to a thermal mass flow controller (Model 840; Sierra Instruments, Amsterdam, The Netherlands) into an air multiplexer (TR-RM8; Sable Systems, Henderson, NV, USA), the other directly into the multiplexer. This design allowed us to direct metered air to a single respirometry chamber for measurement, while simultaneously flushing air through six unmeasured chambers and an empty baselining chamber. In all trials, we set the flow rate of air entering both measured and unmeasured chambers to 600 ml min^{-1}; the flow rate of flush air was established with a bubble meter to ± 10%. We used a Peltier-effect condenser (PC-1; Sable Systems, Henderson, NV, USA) to dehumidify air leaving the measured chamber. Thereafter, we took a subsample of air (150 ml min^{-1}) from the condenser using a 10 ml syringe barrel connected to a subsampler mass flow meter (TR-FC1; Sable Systems, Henderson, NV, USA), which was then connected to a CO_2 analyser (CA-1B; Sable Systems, Henderson, NV, USA). After leaving the CO_2 analyser, the bolus of air was pulled through a series of CO_2 (ascarite) and water scrubbers (drierite) into an O_2 analyser (FC-1B; Sable Systems, Henderson, NV, USA).

During each trial, we programmed our automated respirometry system to measure O_2 consumption and CO_2 production for each bird at 1 s intervals for 10 min per chamber then switch to the next chamber in series. Before beginning measurements on the next bird in the series, we allowed for a 2 min flush to ensure that latent gases had been removed from the system. After the completion of each series of seven 10-min sampling periods, we measured the same gas concentrations in an empty chamber to obtain baseline levels passing through experimental chambers. We then began a new series of seven 10-min sampling periods, repeating this process throughout the night.

By the end of the trial each morning, we had acquired at least six 10-min intervals per bird for the previous night. To determine RMR for each bird during the night, we mathematically identified the 5-min period of lowest O_2 consumption per night for each bird. In all respirometry trials, we used ultra-low permeability Tygon tubing with an internal diameter of 0.32 mm; metabolic chambers were plastic jars (1 litre) with screw-on lids. We surveyed ambient temperature (25 ± 1.5 °C) in the baselining respirometry chamber with an electronic temperature meter (Radio Shack 2100).

(d) *Experimental protocol*

We removed the birds from their cages just before the trials (10 min or less) and transported them to the large respirometry chamber (less than 50 m away) in small cloth bags between 21.00 and 21.30 each evening. We then weighed each bird to the nearest 0.1 g, scored furcular fat (on a 1 to 5 scale) and measured wing-web swelling (mm). We also assessed body temperature (to the nearest 0.1 °C) via a digital thermometer/thermocouple inserted into the cloaca. The above measurements were made in low light (4 W) within 10 min of removal from the cloth bag to ensure that differences in immunological or metabolic parameters were not due to differences in handling times or experimental protocols between groups.

After measurements were made on a bird, we placed it into a respirometry chamber that was placed into a cloth bag and stored on a shelf in a climate-controlled chamber. We repeated this process on the remaining six individuals in random order, and then began simultaneous respirometry measurements of all seven birds until 07.30 the next morning. In the morning, we removed birds from the metabolic chambers and transported them in cloth bags back to their original cages.

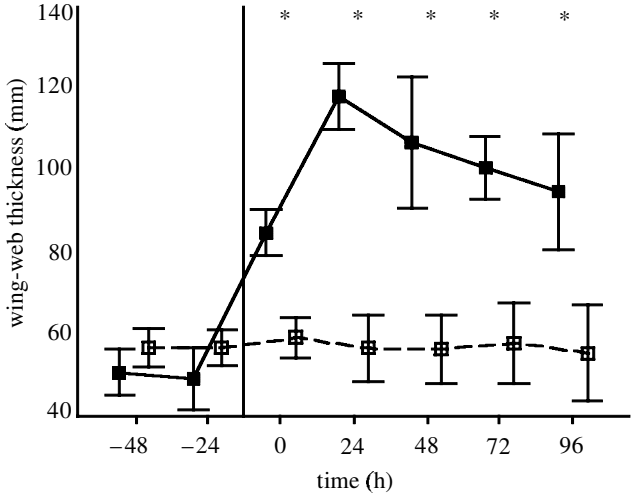

Figure 1. PHA injection into the wing-web of house sparrows caused severe swelling. The solid line indicates PHA-challenged individuals, while the dashed line represents saline-injected birds. Error bars depict 95% confidence interval (CI) of means, and the vertical line indicates time of injection. Asterisks indicate $p < 0.05$ by RM–ANOVA; $n = 7$ for all time points.

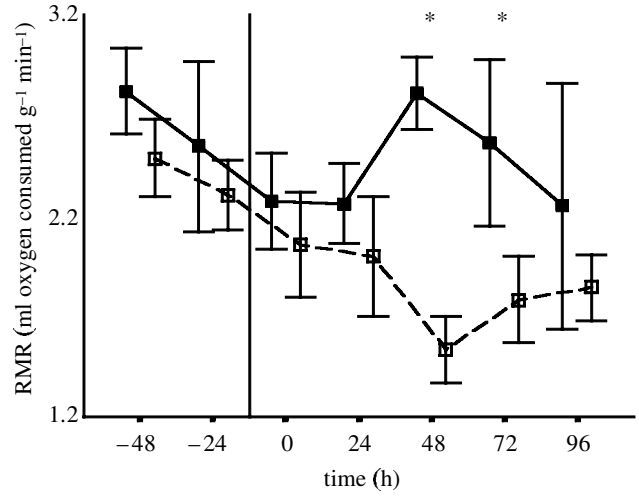

Figure 2. PHA injection significantly elevated RMR of house sparrows relative to controls. The solid line represents RMR values for challenged individuals, whereas the dashed line depicts values for saline-injected birds. Error bars depict 95% CI for means, and the vertical line indicates time of injection. RMR units are ml O_2 consumed $gram^{-1}\,min^{-1}$. Asterisks indicate $p < 0.05$ by RM–ANOVA; $n = 7$ for all time points.

Experimental protocols were approved by the University of Illinois Office of Laboratory Animal Research (OLAR) committee and comply with the 'principles of animal care' (Publ. 86-23, NIH) and current US laws.

(e) *Data analysis*

We used Mann–Whitney *U*-tests to assess differences in initial condition (mass, fat and body temperature) and repeated-measures general linear models (GLM) in SPSS v. 9.0 to assess changes in wing-web thickness and RMR throughout the experiment between PHA-challenged and saline-injected birds. In all GLM comparisons, the effects of body mass on RMR were removed by using the residuals of a linear regression of RMR on body mass; no significant relationship between body mass and PHA response was detected. To examine potential confounds of condition on consequent immune responses or metabolic rates between control and treatment groups, we examined differences in furcular fat scores, mass and body temperature throughout the experiment using repeated measures (RM)–GLM. For all statistical treatments, $\alpha = 0.05$.

3. RESULTS

(a) *PHA challenge*

Injection of PHA significantly increased wing-web swelling of challenged individuals relative to those injected with saline alone (RM–GLM: $F = 62.572$, d.f. = 1, $p < 0.001$). After the initial challenge, mean wing-web swelling of PHA-injected individuals reached levels almost twice as high as for unchallenged individuals, which in turn showed no significant change in wing-web thickness throughout the experiment (figure 1). Swelling in PHA-challenged individuals decreased to pre-injection thickness after *ca.* 10 days (not shown).

(b) *Effects of immune challenge on RMR*

Injection with PHA significantly increased mass-corrected RMR of challenged individuals relative to saline-injected birds (RM–GLM: $F = 37.597$, d.f. = 1, $p < 0.001$). Uncorrected RMR (mass-independent O_2 consumption per bird) showed similar significant results (RM–GLM: $F = 38.696$, d.f. = 1, $p < 0.001$). Initially, there were no differences in RMR between groups; differences between groups did not occur until 48 h after injection and were sustained for the subsequent 24 h interval (from 48 to 72 h of the experiment; figure 2).

(c) *Body mass, fat level and body temperature*

To investigate the effects of initial condition of the two groups of birds, we compared body temperature and furcular fat scores of each individual in the group at the beginning and over the course of the experiment. There were no differences between groups before the experiment (Mann–Whitney *U*-test for body mass: challenged = 28.39 ± 2.29 g, control = 27.96 ± 1.79 g, $p = 0.655$; body temperature: challenged = 41.75 ± 0.81 °C, control = 41.24 ± 1.19 °C, $p = 0.481$; fat: challenged = 1.43 ± 0.79, control = 2.14 ± 0.69, $p = 0.075$), nor were there significant changes in any variable during the experiment (RM–GLM for body temperature: $F = 0.350$, d.f. = 1, $p = 0.565$; fat: $F = 3.78$, d.f. = 1, $p = 0.076$; body mass: $F = 0.086$, d.f. = 1, $p = 0.775$).

4. DISCUSSION

Our results showed that (i) cell-mediated immune activity was metabolically costly for house sparrows, and (ii) this cost was of a sufficient magnitude to affect fitness. Table 1 provides information to put the metabolic costs of immune activity as detected in this study into perspective with other immunological and physiological activities in a variety of passerine species. To establish the total energetic costs of PHA-induced immune activity in our study, we calculated the difference in average metabolic rate between challenged and control birds from the time

Table 1. Energetic cost of immunological and other physiological activity in small vertebrates.

species	body mass (g)	RMR (kJ day^{-1})	physiological activity	duration of measured period (days)	cost per day of measured period (kJ)	cost per day of measured period as percentage of RMR
Passer domesticus[a]	27	14.6	cell-mediated immune response (PHA)	5	4.2	28.8
Parus caeruleus[b]	12	20.4	primary antibody response (DPT)	14	1.6	7.8
			secondary antibody response	7	2.6	12.7
Parus major[c]	19	33.4	primary antibody response (SRBC)	14	2.6	7.8
Mus musculus (C57BL/6J)[d]	36	85.5	primary antibody response (KLH)	14	23.4	27.4
Saxicola torquata rubicola[e]	15	24.7	moult	123	4.2	17.0
S. torquata axillaris	16	22.1		105	3.6	16.3
Carduelis flammea[f]	13	20.8	moult	87	2.7*	13.0
Luscinia svecica	17	22.3		62	6.6*	29.6
Carpodacus mexicanus[g]	20	28.1	testes growth (inactive to maximal size)	40	0.1	0.4
Zonotrichia leucophrys	27	34.9		40	0.2	0.6
Z. leucophrys	27	34.9	ovarian and oviducal growth	20	1.2	3.4
Z. leucophrys	27	34.9	producing eggs	4.7	15.7**	45.0
Troglodytes aedon	12	18.1		6.4	8.8**	48.6
P. domesticus	27	14.6		4.7	8.2**	56.2

[a] Data from this study.
[b] Svensson *et al.* (1998); note that values were statistically insignificantly different from control groups.
[c] Ots *et al.* (2001).
[d] Demas *et al.* (1997).
[e] Klaasen (1995).
[f] Lindstrom *et al.* (1993); *calculated from: cost of molt g^{-1} feathers × feather mass = total cost; per day cost = total cost per average day's molting.
[g] From sources in King (1973); ** estimated from equation in legend of figure 5.

of initial challenge to the end of the experiment. We then summed these differences, transformed this value into litres of O_2 consumed per day and multiplied it by 19.8 kJ, the energy equivalent of 1 litre of O_2 (Gessaman & Nagy 1988; Lindstrom *et al.* 1993). All other values listed in the table were taken directly from the respective studies or calculated using data provided in those studies. Details regarding the calculations of these final values are provided in the table legend.

The data in table 1 indicate that cell-mediated immune activity, and immune activity in general, may impact the energy budget of vertebrates if this activity coincides with other costly physiological processes. For example, the immune response to PHA by house sparrows in our study elevated energy expenditure 4.20 kJ per day during the measurement period. The cost of producing an egg in this species is 8.23 kJ (King 1973). Thus, if cell-mediated immune activity were to coincide with egg production, either reproductive output and/or immune activity may have to be sacrificed if birds cannot increase their daily energy expenditure (Wikelski & Ricklefs 2001; Ricklefs & Wikelski 2002). Competing energy demands may also explain why immune function is often depressed in birds held for short periods at cold temperatures (Svensson *et al.* 1998). Thermogenesis is one of the most energetically demanding activities that passerine birds face; thus birds living under very cold environmental conditions may not be able to balance the energetic demands of both processes (Scholander *et al.* 1950). Physiological processes that take place over longer time-scales, such as moult or growth of reproductive organs, may not be as negatively affected by short-term elevations in energy demands inflicted by induced immune activity. Under such circumstances, the duration of the physiological process at hand may change its time-course.

However, long-term effects of elevated energy expenditure can lead to large fitness costs. At least two studies have shown that elevated energy expenditure covaries with fitness in wild birds. Eurasian kestrels (*Falco tinnunculus*) forced to raise experimentally enlarged broods had significantly decreased survival probabilities relative to birds raising unmanipulated broods. Increased energetic investment by adults in fledgling provisioning led to increased mortality (Daan *et al.* 1996). Similarly, zebra finches (*Taeniopygia guttata*) forced to work at high levels laid eggs six days later than those on low workload levels. In this case, while costs could be recoverable, coincidence with other costly physiological activity (i.e. immune responses)

could delay laying even more, potentially leading to reduced survival of offspring due to late fledging (Deerenberg & Overkamp 1999).

The above studies establishing energy expenditure as a currency for fitness trade-offs also indicate that complete metabolic compensation for an immune challenge might be impossible. In one study, although birds increased their food intake, they were unable to compensate when challenged with SRBCs (Deerenberg *et al.* 1997). Unfortunately, we have no data regarding changes in food intake during this study. Thus, we do not know whether birds tried to compensate for increased energy expenditure due to PHA injection by consuming more food. In the wild, however, such a strategy may be impossible if resource availability fell below a critical level; birds would be immunologically compromised in times of high energy demands, regardless of their propensity to increase feeding rates (Lochmiller & Deerenberg 2000).

Our results, in connection with Daan *et al.* (1996) and Deerenberg & Overkamp (1999), indicate that energetic constraints may have played a part in the evolution of vertebrate immune systems. Furthermore, reduced adult survival and decreased sexual trait elaboration in birds indicate that elevated immune activity (via changes in energy allocation) may be linked to fitness consequences in the long run. It is unlikely, however, that energetic costs are the only relevant costs of immune activity. Nutrient costs may be as similarly important as energetic costs in that limited supplies of a resource might necessitate trade-offs among physiological systems (Klasing *et al.* 1987). Because both types of resources—energy and nutrients—are usually limited, organisms may be forced to down-regulate some physiological activities to upregulate others (Wikelski & Ricklefs 2001). Another factor, immunopathology and autoimmunity, may also have been a major force in the evolution of the vertebrate immune system (Råberg *et al.* 2000). Overactive immune systems may cause substantial harm to organisms, especially during chronic stress (Leclere & Werhya 1989). Although quite different in action, the costs of self-destructive immune activity are no less important than resource-limited costs (Råberg *et al.* 1998).

Our results indicate that cell-mediated immune activity is sufficiently costly to compromise fitness-relevant traits, even if a PHA-induced immune response is only a crude surrogate for a parasite-induced cell-mediated immune response. PHA is widely used in studies of ecological and applied immunology, but it is currently unclear whether subdermal wing-web injections over- or underestimate an average natural cell-mediated response. We suggest that additional tests challenging different aspects of an organism's immune response are needed to understand the full complexity of costs of immune activity in wild vertebrates.

We thank Stefan Klose, Mary Grabowski and Scott Solomon for help with experimentation and care and capture of birds. We thank Michaela Hau, Christiane Meyer, Sara O'Brien, Kevin Rowe, Laura Spinney, Elisa Tarlow and the participants of the 'physiologies of life history' class (summer 2000) at the Research Centre for Ornithology at the Max Planck Society in Andechs, Germany, for comments and creative insight during experimentation and analysis. The University of Illinois, Urbana-Champaign, Princeton University and NSF INT-0000582 supported this work.

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